

Fertile mule in China and her unusual foal¹

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Introduction

The sterility of mules (mare \times jack donkey) has long been recognized. Rare anecdotal reports of fertility in female mules have, however, been made but have not been backed by scientific investigation (Chandley 1981). Nevertheless in China, where mules are bred intensively by artificial insemination, there has been no doubt that the animals are occasionally fertile. The present report describes a fertile mule which gave birth to a filly foal sired by a donkey. Karyotype and biochemical investigations established the authenticity of the case. The foal shows a unique hybrid karyotype not previously described.

Report

A hybrid filly foal was born to a black alleged mule on 4 March 1981 in Tielu production brigade, Yancheng Count, Henan Province, China (Figures 1 and 2). A feeder, who was one of two eye-witnesses to the birth, stated that the mother had had regular oestrous cycles at 18-day intervals. It was concluded that the sire was a donkey who had, over a long period of time, been stabled with the mule. He, however, had been sold and could not now be traced. Blood samples for karyotyping were collected soon after the birth from both mother and offspring, now named 'Dragon Foal', and repeat samples were taken at subsequent intervals. Blood samples for serum cholinesterase analysis by polyacrylamide gel electrophoresis were also collected from the mother and foal, and from several horses, mules, hinnies and donkey controls.

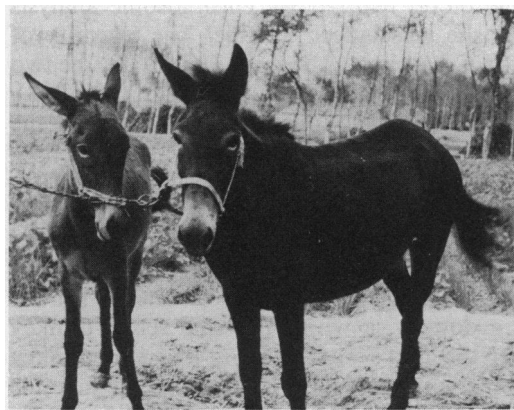


Figure 1. The foal (aged 1½ years) with its mule mother

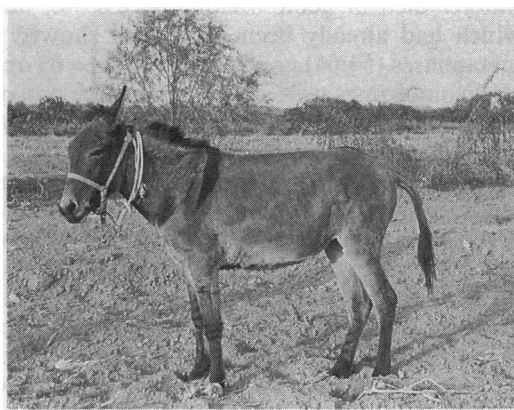


Figure 2. The foal aged 4 years

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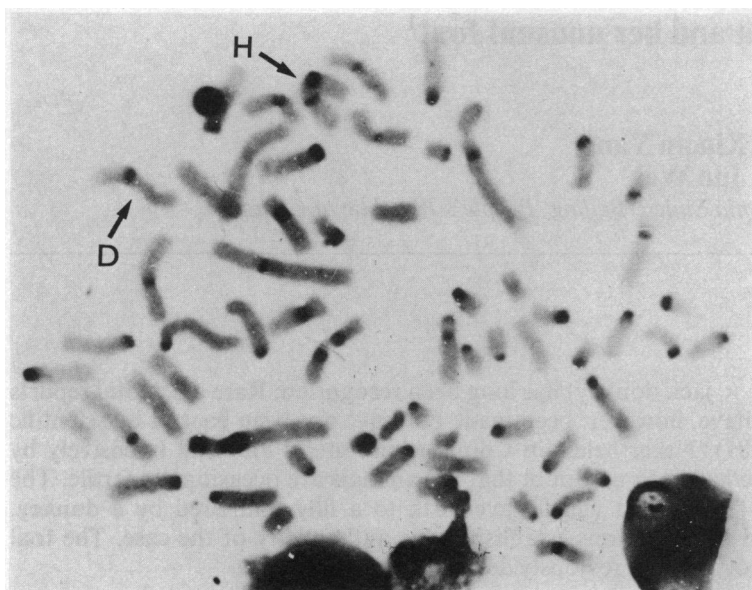


Figure 3. C-banded blood lymphocyte metaphase from the mule mother. (H = horse X, D = donkey X)

Phenotypically, the foal appears to be a fascinating mix of horse, donkey and mule-like features. An independent opinion, sought from L Travis, Secretary, British Mule Society, assessed the foal's appearance in several photographs, including Figures 1 and 2, as follows:

The tail is set very low and resembles that of a donkey. The legs, both in shape and striping, are also donkey-like (stripes are fairly common in donkeys but virtually non-existent in horses). The body appears too deep for a donkey, but the hind-quarters are donkey-like, the slope from the tail to the top of the legs being donkey-shaped. (Most mules have donkey-like hindquarters but this foal is more donkey-like than most mules.) The shoulders and neck, however, are horse-like and too well built for donkey or most mules. The head and ears are mule.

'Dragon Foal' continues to thrive, and at 4 years of age (Figure 2) is strong enough to plough a field by herself. She now also shows strong oestrous behaviour. In 1983 the mother died of acute abdominal disease, but the blood lymphocyte chromosome analysis which had already been performed showed that she was indeed a mule. The majority of metaphases (54/64) gave a count of $2n=63$ and, on C-banding (Arrighi & Hsu 1971), the two X-chromosomes were clearly seen to be one of horse, the other of donkey, origin (Figure 3). Additional and unequivocal evidence of her hybrid chromosome constitution was found in the autosomal complement.

The karyotype of 'Dragon Foal' gave a modal number of $2n=62$ (105/115 metaphases analysed), the same count as that for the donkey (Ryder *et al.* 1978). The C-banded karyotype is shown in Figure 4. The total number of acrocentrics was 24 (or 25), a number intermediate between donkey (22) and mule (30). Two clear donkey X-chromosomes were present. Also present were a number of autosomes and pairs of autosomes which were distinctive for the donkey. As the sire was believed to have been a donkey, a whole paternal set of donkey chromosomes was assumed, but the presence in the foal's karyotype of *pairs* of donkey chromosomes shows that donkey chromosomes must also have been contributed by the mule mother. For example, line 1 in the C-band karyotype of Figure 4 shows the largest pair to be a chromosome common to both horse and donkey (identification confirmed on G-banding). The next five chromosomes in the line, each present singly, are all distinctively donkey. The eighth and ninth chromosomes in this line are probably a pair (unless one of them is a pair with the tenth). In any case, a chromosome pair can be formed from these three chromosomes and, as the horse karyotype does not contain a metacentric chromosome showing C-bands at

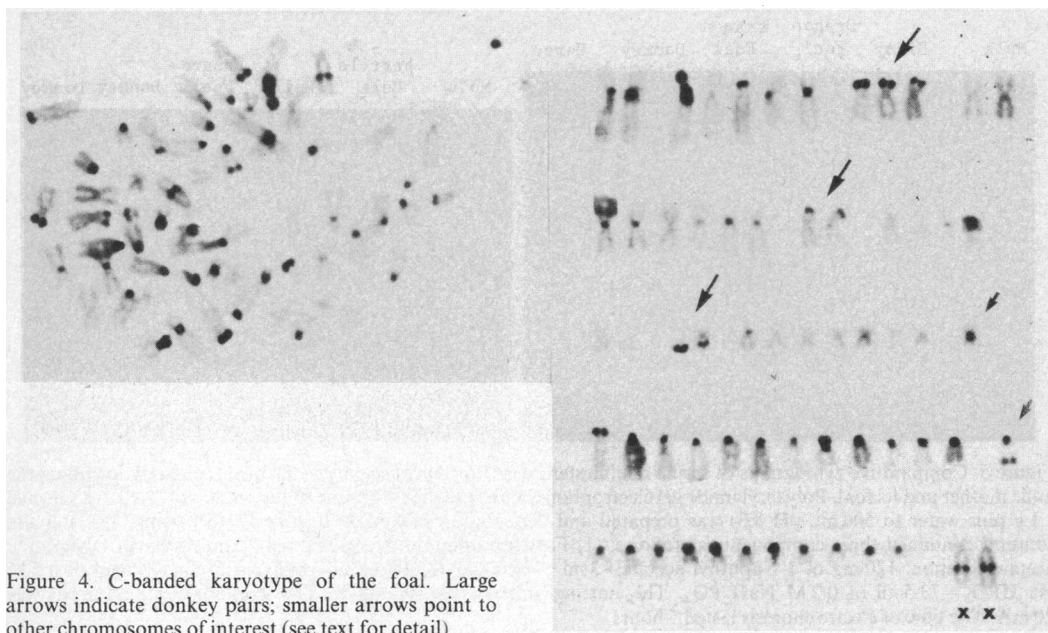


Figure 4. C-banded karyotype of the foal. Large arrows indicate donkey pairs; smaller arrows point to other chromosomes of interest (see text for detail)

the distal tips of its short arm (Buckland 1976), this must be a donkey pair. The same is true of the seventh and eighth chromosomes in the second line showing C-bands at the tips of the short arms, and also of chromosomes 3 and 4 on line 3. At the end of line 3, there is a small metacentric with a centromeric C-band. The donkey karyotype has no such chromosome (Ryder *et al.* 1978); this is probably horse No. 12 (Buckland *et al.* 1976, Ford *et al.* 1980). From the C-band karyotype alone, it can thus be concluded that the mother mule contributed both horse and donkey chromosomes to the foal via her ovum.

Unfortunately, G-banded chromosome preparations have not been produced where every chromosome can be unequivocally identified as either donkey or horse in origin, or common to both species. Preliminary G-banding studies do, nevertheless, indicate about eleven matching pairs of chromosomes. Some of these, as mentioned earlier, are purely donkey pairs. Some, for example the two largest metacentrics, occur in both the donkey and horse karyotypes and although one chromosome represents a donkey chromosome contributed by the father, the other could have originated from either the horse or donkey components of the mule mother. Other chromosomes, in particular four submetacentrics, were horse chromosomes with no equivalents in the donkey karyotype. So these can only have come from the mother.

The results of the serum cholinesterase study showed that the zymograms in horse, mule and hinny were similar to each other, but different from the donkey. The major difference was the two dark-stained bands which are the fastest-moving in the gel, and this difference was clear and unequivocal (Figure 5). The zymogram of the foal was identical to those of horse, mule and hinny (as was the fertile mule mother) rather than to that of the donkey.

Discussion

From the work of Taylor & Short (1973), it is known that the germ cells migrate normally into the fetal gonads of female mules and surviving oocytes will induce the development of normal follicular cells. The ovary is capable of acquiring some endocrine activity in later life (Taylor & Short 1973) and she-mules may come into oestrous at irregular intervals (Bielanski 1972). The vast majority of oocytes, however, fail at meiosis owing to the pairing problems encountered when the structurally different horse and donkey chromosome sets attempt to synapse at meiotic prophase. Anderson (1939) advanced a hypothesis that the ova of she-mules which

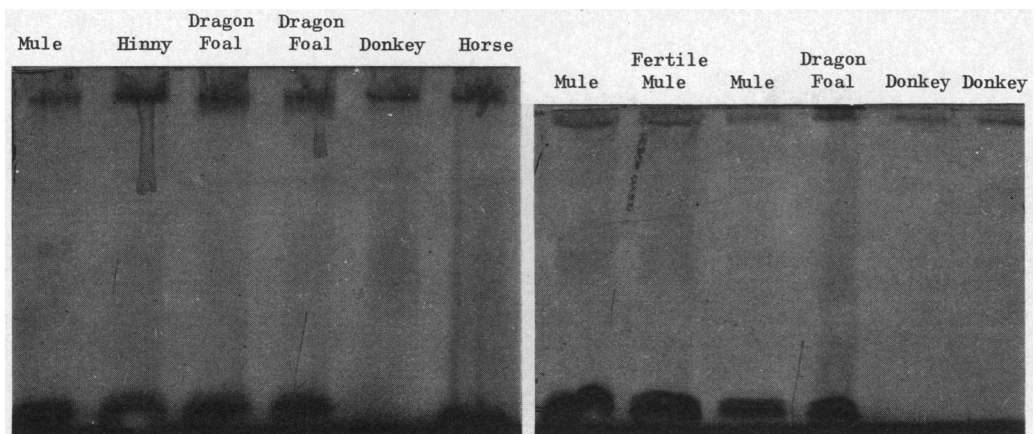


Figure 5. Comparative zymograms of serum cholinesterase electrophoresis analysis in various equines, including the mule mother and its foal. Polyacrylamide gel electrophoresis was used. Tris-glycine buffer solution (Tris 3.1 g, Glycine 3.1 g plus water to 500 ml, pH 8.3) was prepared and the solution diluted to 10 times before using. The staining solution contained three components as follows: (1) Fast red solution: 50 mg fast red + 5 ml H_2O ; (2) 1-Naphthyl acetate solution: 120 mg of 1-Naphthyl acetate + 3 ml of acetone; (3) Phosphate buffer solution: 26.5 ml of 0.2 M Na_2HPO_4 + 73.5 ml of 0.2 M NaH_2PO_4 . The starting voltage was 100–140 V. The working electric current was 20 mA. The time of electrophoresis lasted 5 hours

produce viable pregnancies carry only horse chromosomes, while those of the hinny carry only donkey chromosomes. This idea was extended by Chandley (1981) into the 'affinity' hypothesis. The results of the karyotypic analysis on 'Dragon Foal' indicate, however, that Anderson's (1939) hypothesis, or that of Chandley (1981), cannot always apply. In our foal, the mother mule has provided an ovum with a mixture of horse and donkey chromosomes. Although the count and sex chromosome constitution was the same as for the donkey, the autosomal complement was clearly of mixed origin. The proportional mix of horse and donkey chromosome was, however, different from the 1:1 ratio found in the mule and hinny, there being a higher proportion of donkey chromosomes. There was, therefore, less deviation from a full parental donkey genome than in the case of the mule and hinny.

The exact make-up of the autosomal complement of the foal remains to be determined, but enough can be seen from the investigation so far to conclude that our fertile mule has given birth to some completely new kind of hybrid animal, never before described in the scientific literature. Phenotypically, she appears to show a mixture of horse, donkey and mule-like characteristics quite consistent with the karyotypic findings. That such an odd combination of horse and donkey chromosomes could produce a viable offspring of sound constitution is remarkable. It endorses previously held beliefs that a good deal of genetic homology probably remains between the horse and donkey in spite of the karyotypic changes which have taken place over evolutionary time between the two species (Ryder *et al.* 1978, Chandley *et al.* 1974). It would seem that all the correct and necessary genetic material has been inherited by our hybrid foal, giving it a harmoniously functioning, if unusual, genome to ensure development and survival. It will be of utmost interest to test the fertility of the foal. If some female mules are fertile, there is a much better chance that this animal, which has a less severe departure from a balanced parental (donkey) genome, would be fertile too. Other novel combinations of horse and donkey chromosomes might then be found among hybrid offspring produced.

Addendum

Since this investigation began, another scientifically authenticated case of mule fertility has been recorded (Ryder *et al.* 1985). In the USA, a female mule mated to a jack donkey has produced a colt foal which, on karyotyping, has proved chromosomally to be a mule. In this

case, it would seem that a full horse complement passed into the ovum at meiosis in the mother mule, thus obeying the 'affinity' rules.

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